Hyperplasia Induced by a Δ^{16} Steroid.* (29079)

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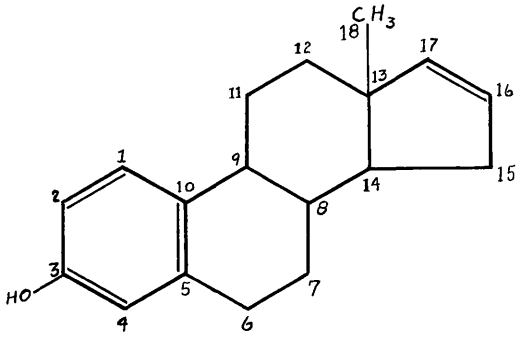
For the last 10 years the author has been using fish embryos, *Brachydanio rerio* (Hamilton-Buchanan), as a means of screening chemicals that affect mitosis and/or embryonic differentiation(1). Among the chemicals tested was 1, 3, 5 (10), 16 estratetraen-3-ol (2) (hereinafter coded and referred to as



Structural formula 1, 3, 5 (10), 16 estratetraen-3-ol (#742)

#742). This particular chemical (first tested by us in 1957) was found to be *not* cytostatistically active during early cleavage. However, it did cause abnormal development and hyperplastic growth after organogenesis had been well established. In dilute solutions (0.5 ppm) this activity seemed to be localized in the tip of the tail (Fig. 2). Because of the constancy and specificity of effect various studies were made using the fish and other embryos as test materials.

Materials, methods and results. The delta-16-steroid #742 was synthesized by the chemists at the Lasdon Foundation at Colorado Springs(2) and supplied for testing dissolved in propylene glycol 1.0 mg/ml.

Eggs of *B. rerio* were obtained(1) and exposed at different stages of development by placing them in 50 ml of culture water to which the appropriate amount of #742 had been added. Embryos were cultured at 80°F ± 1° (26.5°C). Controls on both water and propylene glycol were provided in each test made.

In concentrations of 2 ppm of #742, all embryos (100%) developed necrotic tail tips (Fig. 2) when about 24-36 hours of age (tail free stage when tail movement and circulation begins). Activity of the drug, as expressed by hyperplasia in the tail region of some embryos, has been detected in concentrations as low as 0.1 ppm, consistently at 0.5 ppm. No tumors or similar necrotic conditions have ever been observed in the controls either in water or in propylene glycol as high as 2%.

More than 100 experiments in replicates involving several thousand embryos have given consistently similar results. It apparently makes little difference whether the drug is administered during early cleavage or after 24 hours of development. It should be administered before the fin rays start to develop and before circulation in the tail is well established.

At higher concentrations, 2 ppm and up, there usually occur stoppage of tail development and/or growth as well as necrosis of the epidermis over the entire body. Sometimes malformations appear visible in other parts of the body (Fig. 4). Embryos usually die by the end of the fourth day (96 hours).

In lower concentrations, 0.5-1.0 ppm, the first visible effect is cellular abnormalities at the end of the notochord, tip of the tail, followed by hyperplasia (Fig. 2). Necrosis of the tail progresses and eventually death occurs.

During the springs of 1960 and 1961, eggs and larvae of *Rana pipiens* were collected and exposed to #742 in concentrations varying from 1.0 to 5.0 ppm. The higher concentrations were lethal but the lower concentrations caused malformations not only in the tail but elsewhere (Fig. 3 and 4), when embryos were exposed prior to the closure of the neural tube. Newly hatched larval forms

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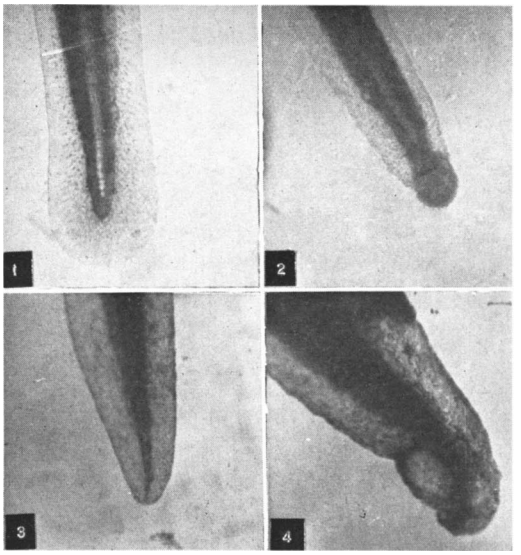

FIG. 1. Normal—fish embryo tail *Brachydanio rerio.*
FIG. 2. Treated, 0.5 ppm—fish embryo tail *Brachydanio rerio.*
FIG. 3. Normal—tadpole tail *Rana pipiens.*
FIG. 4. Treated, 1 ppm—tadpole tail *Rana pipiens.*

and older tadpoles (*Rana,* various species) did not respond to concentrations of 1.0 and 0.5 ppm. One test on tadpoles (newly hatched), *R. pipiens,* at 2.0 ppm resulted in bizarre epithelial growths at the tip of the tail which were eventually absorbed. Older animals and tadpoles that had recovered from treatment would not respond to concentrations of 3.0 and 5.0 ppm.† Kivett(3) tested #742 on chick embryos and obtained evidence of tumor formation.

Discussion. From our studies it appears obvious that #742 produces hyperplasia in some amphibian and teleost embryos when applied during the development of the tail fin. The exact nature of the reaction and the histology involved are not understood. Studies involving tissue cultures and cytological effects will be reported later.

The fact that a delta-16-steroid consistently produces hyperplasia in the same developmental region and stage at relatively low concentrations (0.5 ppm) seems to be significant.

Summary. Embryos of *Brachydanio rerio* and *Rana pipiens* when grown in water containing 0.5-2.0 ppm of 1, 3, 5 (10), 16 estratetraen-3-ol (a Δ^{16} steroid) will consistently develop malformations (hyperplasia) in the region of the tail if exposed before the formation of the tail fin occurs.

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† Appreciation is expressed to members of the classes in Embryology at Oklahoma State Univ. who conducted studies on the effects of #742 on amphibian embryos.

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2. Huffman, M. N., Lott, Mary Harriet, Tillotson, Albert, *J. Biol. Chem.,* 1955, v217, 103.
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Anti-Androgenic Activities of A-Nortestosterone and C-Nor-D-Homo-17α-Epitestosterone Acetate. (29080)

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Structural alteration of naturally occurring steroids may lead to compounds with interesting biological properties. Reduction of the 6-membered A-ring of progesterone to the 5-membered A-ring of A-norprogesterone(1) yielded a compound without progestational activity but with the property to antagonize the effects of exogenous and endogenous androgen at specific end organs(2,3,4). The A-nor analogue of testosterone, A-nortestosterone(1), is a very weak androgen and is anti-androgenic. These properties have been